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Effects of capture on stress-axis measures in endangered little brown bats (*Myotis lucifugus*)

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Little brown bats (*Myotis lucifugus*) are a widely distributed species in North America that have been decimated by the fungal disease white-nose syndrome. As such, little brown bats are the focus of monitoring and research initiatives that often include capturing and handling free-ranging individuals. We examined the stress response of 198 adult female little brown bats after being captured from three bat houses, during the summer. Our objective was to inform best practices to researchers capturing and handling bats in the wild. We compared the stress response among bats held for <3 min (baseline), 15–30 min, or >30 min, and then among bats held alone or in a group with conspecifics. We measured the levels of plasma total and free cortisol, maximum corticosteroid binding capacity (MCBC), and blood glucose. Relative to baseline, total and free cortisol levels were significantly higher in bats held for 15–30 min and higher still in those held for > 30 min. Blood glucose levels were elevated after >30 min of holding. MCBC levels showed no differences among holding times. We detected a weak effect of social holding condition, with solitary-held bats having lower total cortisol levels than group-held bats, but MCBC, free cortisol, and blood glucose levels showed no effect of social holding condition. Our findings demonstrate that capture time should be minimized and suggest that little brown bats should be handled and released within 30 min of capture as means of reducing stress. Further, solitary holding did not appear to increase stress measures, which supports holding bats individually after capture, instead of in groups, to reduce risk of pathogen and parasite transmission.

Key words: animal welfare, conservation physiology, Chiroptera, hormone assay, sociality, stress, wildlife capture

Little brown bats (*Myotis lucifugus*) are one of the most widespread bat species in North America, with a range encompassing much of Canada below the treeline, the continental United States, and a portion of northern Mexico (Fenton and Barclay 1980). Since 2012, they have been listed as endangered under Canada's Species at Risk Act due to the devastating effects of white-nose syndrome (WNS; COSEWIC 2013). White-nose syndrome is a fungal infection that was discovered in the northeastern United States in 2006 and since has caused the collapse of a number of bat populations in North America, with little brown bats being the most heavily affected species (Frick et al. 2010, 2016, 2017; Vanderwolf and McAlpine 2021). The white-nose fungus (*Pseudogymnoascus destructrans*) grows optimally at cool temperatures and affects bats during their winter hibernation, invading their cutaneous tissue and causing downstream effects that lead to awaking during hibernation, depleting fat reserves which are necessary for overwinter survival (Reeder et al. 2012; Warnecke et al. 2012, 2013; Frick et al. 2016). In the time since this disease was initially detected, and when little brown bats were listed as endangered in Canada in 2012, it had caused a 94% decline in eastern Canadian populations of the species (COSEWIC 2013). In response, recovery experts designated population monitoring and research on little brown bats a high priority in the species' recovery strategy (Environment and Climate Change Canada 2018). As such, the capture, handling, and

biological sampling of little brown bats will remain necessary (e.g., Hooper and Amelon 2014; Environment and Climate Change Canada 2018). Important work is ongoing both in understanding and treating white-nose syndrome in bats (e.g., Verant et al. 2014; Davy et al. 2017; Hyot et al. 2019), and ensuring that monitoring by researchers does not adversely affect their populations (Kilpatrick et al. 2020).

Live capture of wild animals and other stressors result in the activation of hypothalamic-pituitary-adrenal (HPA) axis and secretion of glucocorticoids (GCs; primarily cortisol or corticosterone depending on the species). Within minutes of the perception of a stressor, GCs are released from the adrenal cortex into circulation. These hormones trigger physiological and behavioral changes that allow animals to adaptively respond to a threat, including glucose mobilization, breakdown of stored energy reserves, decreased appetite and feeding behavior, and increased vigilance (Sapolsky et al. 2000). There also can be changes in the concentration of the major GC carrier protein, corticosteroid-binding globulin (CBG). In mammals, ~90% of GCs are bound to CBG, with the unbound portion ("free GCs") being able to cross cell membranes to interact with receptors (Breuner et al. 2013). The CBG-bound portion of GCs is thought to act as a reservoir and CBG may also act as an additional modulator of GC activity (Rosner 1990; Boonstra 2005; Breuner et al. 2013). Longer exposure to stressors will reduce levels of CBG (though not in all species), consequently "freeing" more GCs, but the timescale for this effect tends to be slower than changes in GC secretion, and occurs after several hours (Marti et al. 1997; Delehanty and Boonstra 2009). In contrast, GCs are secreted in response to an acute stressor within minutes. GC secretion does not persist indefinitely after an acute stressor and is regulated via negative feedback where GCs bind to glucocorticoid receptors in the hypothalamus, hippocampus, and pituitary and inhibit the stress-axis (Redei et al. 1994; Boonstra 2005).

In live-trapped animals, the prevailing advice is that blood samples collected in ≤ 3 min represent baseline (unstressed or "true base") GC levels, and samples afterwards will show GC elevation as a result of trapping stress. This "3-minute rule" has generally been accepted across studies (e.g., Boonstra 2005; Romero and Reed 2005; Small et al. 2017; Lawrence et al. 2018). In little brown bats, Reeder et al. (2004) demonstrated that bats restrained for 15 min had significantly higher total cortisol levels than those sampled in ≤ 3 min. Similarly, in variable flying foxes (*Pteropus hypomelanus*) those handled for 15 min had higher cortisol levels than those handled for ≤ 3 min (Widmaier et al. 1994). Hence, the literature predicts that capture would increase cortisol levels in little brown bats. Yet there are fewer available data on whether stress hormones and proteins plateau following prolonged confinement. In the stress-response, maximum GC secretion is reached within 15 to 30 min, after which negative feedback reduces GC levels (Sheriff et al. 2011). However, GC secretion depends on the magnitude and length of the stressor; if the stressor is persistent, GC secretion can be sustained (Sheriff et al. 2011). For example, in meadow voles (*Microtus pennsylvanicus*), live-trapping increased plasma corticosterone levels, but the magnitude of the response was the same whether the voles had been in traps for 2 h or for up to 9 h (Fletcher and Boonstra 2006).

Social holding conditions during capture can also affect GC levels, but these effects are unknown for little brown bats. Little brown bats were often held in bags or other holding devices with several conspecifics after being live captured and prior to being handled and released; however, researchers are likely moving toward holding captured bats alone to reduce pathogen and parasite transmission. The effects of social housing on stress-measures are species specific, based on how the conditions relate to the species ecology and sociality (Beery et al. 2020). For example, in mink (*Neovison vison*), which are naturally solitary, captive group housing resulted in higher cortisol levels than single housing (Hansen and Damgaard 1991). Conversely, in captive populations of a naturally social and colony-living species, the naked mole-rat (*Heterocephalus glaber*), simply removing an individual from the colony increased its cortisol levels (Edwards et al. 2020). Similarly, in group-living sheep (*Ovis aries*), removal from the group to solitary pens elevated cortisol levels (Guesdon et al. 2015). Little brown bats often roost in groups; in the summer, adult females aggregate in maternity colonies of up to hundreds of individuals and, in winter, males and females may aggregate by the thousands for hibernation (Fenton and Barclay 1980). Therefore, they may benefit from being held with conspecifics to ameliorate the stress of capture. However, even for species that are naturally social or socially tolerant, being held with conspecifics that are not necessarily members of the same natural social group or colony could potentially be stressful because established social dynamics or hierarchies may be disrupted (Wilcox and Willis 2020). Furthermore, in an enclosed area, individuals have no ability to escape from potential aggressors.

Our aim was to test the hypothesis that duration of holding between capture and release, and social conditions in which bats are held, affect measures of the stress-axis. In addition, we sought to better understand if blood samples collected within different time-frames are comparable in stress-axis measures. Following from Reeder et al. (2004), we expected that capture would increase cortisol levels in little brown bats. However, we investigated whether this effect plateaus, or if 15–30 min and >30 min holding times differed. We compared bats that were captured and bled immediately (< 3 min; baseline) with those held for 15–30 min and those held for > 30 min. We also compared their ability to mobilize energy (glucose levels) at these different times. Glucose mobilization often is used as a measure of stress-axis activation because glucocorticoids elevate blood glucose levels by converting lipids to glycogen, catabolizing body reserves, and increasing hepatic glucose synthesis (Wingfield et al. 1998; Boonstra et al. 2001; Malisch et al. 2018). This is thought to be a process in the stress response with adaptive value, as the energy can be used in escape behavior or other survival processes (Sapolsky et al. 2000).

To assess the effect of social holding conditions, we compared stress-axis measures in little brown bats held in groups

after capture to those held alone. We expected that being held either alone or with conspecifics for ≥30 min prior to handling and release the same evening could affect stress-axis measures, but we could not predict the directionality of the effect given their colonial nature. The impetus of our work was to inform best practices for capture and handling of little brown bats to minimize stress.

Materials and Methods

Bat capture and plasma collection

Little brown bats were captured from three maternity colonies in southern Yukon, Canada; specifically: Squanga Lake (60.448°N, −133.603°W), Salmo Lake (60.447°N, −133.564°W), and Little Atlin Lake (60.281°N, −133.970°W), approximately 55–70 km northwest of the Village of Teslin (Slough and Jung 2008, 2020). The size of each colony varied from about 120 – 250 adult females, as determined from annual emergence counts. Maternity colonies occurred in large bat houses (approximately 120 × 90 × 20 cm) provided as supplementary roost sites > 6 years prior to our captures (Slough and Jung 2020). At Little Atlin Lake, bats also resided in the shed to which the bat house was affixed. We used homemade 2-bank harp traps (Tuttle 1974; Francis 1989) hung below bat houses to capture bats as they emerged shortly after sunset (Fig. 1).

To investigate effects of time since capture on GCs, bats were captured on 27–30 May and 12–14 June 2013 at each colony. Once captured, we determined the sex and age-class of the bat, measured its mass (± 0.1 g), assessed its reproductive status and, if not already banded, we affixed an individually marked 2.9 mm internal diameter wing band (Porzana Ltd., Icklesham, United Kingdom) prior to releasing it on site. Sex was determined by presence of a penis, and we distinguished juveniles from adults by the ossification of epiphyses in the metacarpophalangeal joints, via transillumination of the wing (Kunz and Anthony 1982). Only adult females were used in this study. We determined whether adult females were pregnant by gently palpating the abdomen to feel for a fetus. In the Yukon, female little brown bats typically are in early pregnancy during these periods, and 88% are pregnant (Slough and Jung 2008). We removed bats to be sampled and placed them individually in cloth holding bags labeled with the time of capture until they could be processed. To provide a sample of bats that were bled at baseline, we processed some bats as soon they emerged and fell into the harp trap. We used a 26- or 27-gauge needle to puncture the interfemoral vein, located in the uropatagium, of captured bats and attempted to collect 50–75 µl of serum with a 100-µl heparinized hematocrit tube, following Eshar and Weingerg (2010). When we were unable to draw serum from the interfemoral vein, we also tried the cephalic vein, located along the leading edge of the propatagium. Capillary tubes were immediately flame-sealed and dipped in sealing wax. Volumes of blood we collected represent ≤ 1% of the total volume of blood in little brown bats (Eshar and Weingerg 2010; Hooper and Amelon 2014). A drop of blood was immediately used to measure blood glucose using a portable blood glucose meter (My FreeStyle; Abbot Laboratories, Alameda, California). Blood samples were centrifuged within 6 h to separate plasma, which then was frozen at −20°C until hormone analyses. We collected blood from 35 adult female bats at baseline, 25 bats in 15–30 min, and 14 bats in > 30 min, after capture.

Fig. 1.—Photograph of a bat house in southcentral Yukon, Canada, that housed a maternity colony of little brown bats (*Myotis lucifugus*) that were sampled in this study. Hanging below the bat house is a homemade harp trap that was used to capture bats as they exited the house (see Material and Methods for details). For scale, the ladder is 366 cm long.

To investigate the effect of solitary versus group holding conditions on stress-axis measures, bats were captured during two capture sessions: early July (3–8 July) and late July (17 July to 7 August). Because there was only a single August sampling day (7 August; n = 19 animals captured), we grouped these samples into the "late July" treatment. We focused our sampling on reproductively active adult females. Little brown bats in our colonies give birth in late June to early July (Slough and Jung 2008), and we sampled only females in late pregnancy during the early July capture sessions. By late July females at our colonies are typically lactating, and by early August they are post-lactating, so we sampled only females that were lactating or post-lactating. Bats were held after capture either individually (hereafter, solitary) or in groups of 8–10 animals (hereafter, group), in bags hung on a line ≥ 25 m away from bat houses. Bats were placed in bags in ≤ 15 s after falling into the harp trap. We drew blood from 56 solitary and 68 group-held adult female bats ≥ 30 min after capture, using the procedures

described above. After venipuncture, we applied gentle pressure to the puncture site until the bleeding completely stopped to encourage hemostasis of the punctured blood vessels (Eshar and Weingerg 2010).

Hormone analysis

Cortisol is the primary glucocorticoid in little brown bats; corticosterone only accounts for on average 5% of their total glucocorticoids (Reeder et al. 2004). Plasma total cortisol levels were measured using a commercially available ^{125}I radioimmunoassay (MP Biomedicals, Santa Ana, California). Cross reactivities of the antiserum for this kit are reported as corticosterone 5.5%; prednisolone 45.6%, cortisone 2.1%, 11-desoxzycortisol 12.3%, and progesterone 0.25%. Total cortisol was determined in two assay runs, with an inter-assay coefficient of variation (*CV*) of 1.5% and an intra-assay *CV* of 4.9%. Samples were run in duplicate, using 3 µl of bat plasma per tube. To determine plasma CBG levels, the maximum corticosteroid-binding capacity (MCBC) was quantified by using dextran-coated charcoal to separate bound hormone (Delehanty and Boonstra 2009; Delehanty 2015). The intra-assay *CV* for the MCBC assay was 9.3%. Free cortisol levels then were calculated using the equation developed by Barsano and Baumann (1989) using the species-specific equilibrium dissociation constant (K_d) of 0.75 nM (Desantis et al. 2013).

Data analysis

Data were analyzed using R version 3.6.3 (R Core Team, 2020). Linear mixed effect models (LMMs) were built using the package "nlme" (Pinhiero et al. 2020). Total cortisol, MCBC, and free cortisol were log transformed so that model residuals approximated normality. The glucose data had normally distributed residuals so they were not transformed. Response variables were total cortisol (log ng/ml), MCBC (log ng/ml), free cortisol (log ng/ml), or glucose (mmol/l) concentration. For the time since capture data, LMMs were built treating time since capture and capture date (late May or mid-June) as fixed effects. Reeder et al. (2004) reported an effect of reproductive status and phenology on total cortisol levels in little brown bats, so we examined the effect of capture date on these metrics. Colony (Squanga or Salmo) was included as a random effect. In these models, comparisons of capture time were relative to the baseline group as the intercept, so we subsequently compared the 15–30 min group and the > 30 min group with post-hoc comparison tests (i.e., Tukey Honestly Significant Difference), using the "emmeans" package (Lenth 2020).

To test for an effect of social holding after capture, LMMs were built with total cortisol (log ng/ml), MCBC (log ng/ml), free cortisol (log ng/ml), or glucose (log mmol/l), as response variables. In this dataset, glucose concentration also had to be log transformed to approximate normality. Holding condition (solitary or group) and capture date (early July or late July) were included as fixed effects, and origin colony (Squanga or Salmo) as a random effect. A single outlier was removed from this dataset, which had free cortisol value recorded as 3 ng/ml and subsequently a negative free cortisol value. This value was far below the range of total cortisol values (70–1982 ng/ml), and only 0.6% of the mean; therefore, we assumed this was a measurement error.

Results

In total, we collected plasma in the field from 198 individual female little brown bats. We obtained adequate volumes of serum (20–75 µl) from most bats to determine glucose (n = 196; 99%), total cortisol (n = 174; 85%), MCBC (n = 168; 85%), and free cortisol (n = 163; 82%). We were unable to draw any blood from two individuals.

Relative to the baseline group, total and free cortisol levels were elevated at 15–30 min ($P < 0.001$ for both measures) and at > 30 min ($P < 0.001$ for both measures) after capture (Table 1; Fig. 2). We then compared the 15–30 min and the > 30 min groups with a Tukey HSD test. Relative to the 15–30 min group, mean total cortisol levels were 1.5 times higher in the > 30 min group, but there was no statistically detectable difference in total cortisol levels between 15–30 min and > 30 min (t = 2.14, P = 0.09; Fig. 2A). MCBC levels did not significantly differ among any of the time points from (baseline vs. 15–30 min, P = 0.62; baseline vs > 30 min, P = 0.23; 15–30 min vs > 30 min, P = 0.77; Fig. 2B). Relative to the 15–30 min group, mean free cortisol levels were 2.3 times higher in the > 30 min group and a significant increase in free cortisol levels was detected (t = 3.73, P < 0.01; Fig. 2C). Blood glucose levels did not increase between the ≤ 3 min of capture and 15–30 min of capture groups (P = 0.22); however, by > 30 min of capture, they had increased to 1.9 times baseline levels (P < 0.001; Fig. 2D). In all four models, sampling date had no effect on the response variable (P > 0.66).

Table 1.—Model output of linear mixed effect models testing the effects of capture time and sampling date on stress-axis measures of adult female little brown bats (*Myotis lucifugus*) from Yukon, Canada. Time of capture is relative to baseline as the intercept. Sampling date are mid-June, relative to late May as the intercept. Bat origin colony was used in all models as a random effect.

Response variable	n	Parameter	Estimate ± SE	t value	P value
Total cortisol (log ng/ml)	58	15–30 min	0.44 ± 0.06	7.28	< 0.001
		>30 min	0.62 ± 0.07	8.55	< 0.001
		Sampling date	–0.02 ± 0.06	–0.40	0.69
MCBC (log ng/ml)	52	15–30 min	–0.05 ± 0.10	–0.50	0.62
		>30 min	–0.14 ± 0.11	–1.22	0.23
		Sampling date	–0.03 ± 0.10	–0.27	0.79
Free cortisol (log ng/ml)	52	15–30 min	0.94 ± 0.12	7.85	< 0.001
		>30 min	1.52 ± 0.14	11.12	<0.001
		Sampling date	0.02 ± 0.12	0.18	0.86
Glucose (mmol/l)	72	15–30 min	0.58 ± 0.47	1.23	0.22
		>30 min	3.09 ± 0.62	4.98	<0.001
		Sampling date	0.21 ± 0.48	0.44	0.66

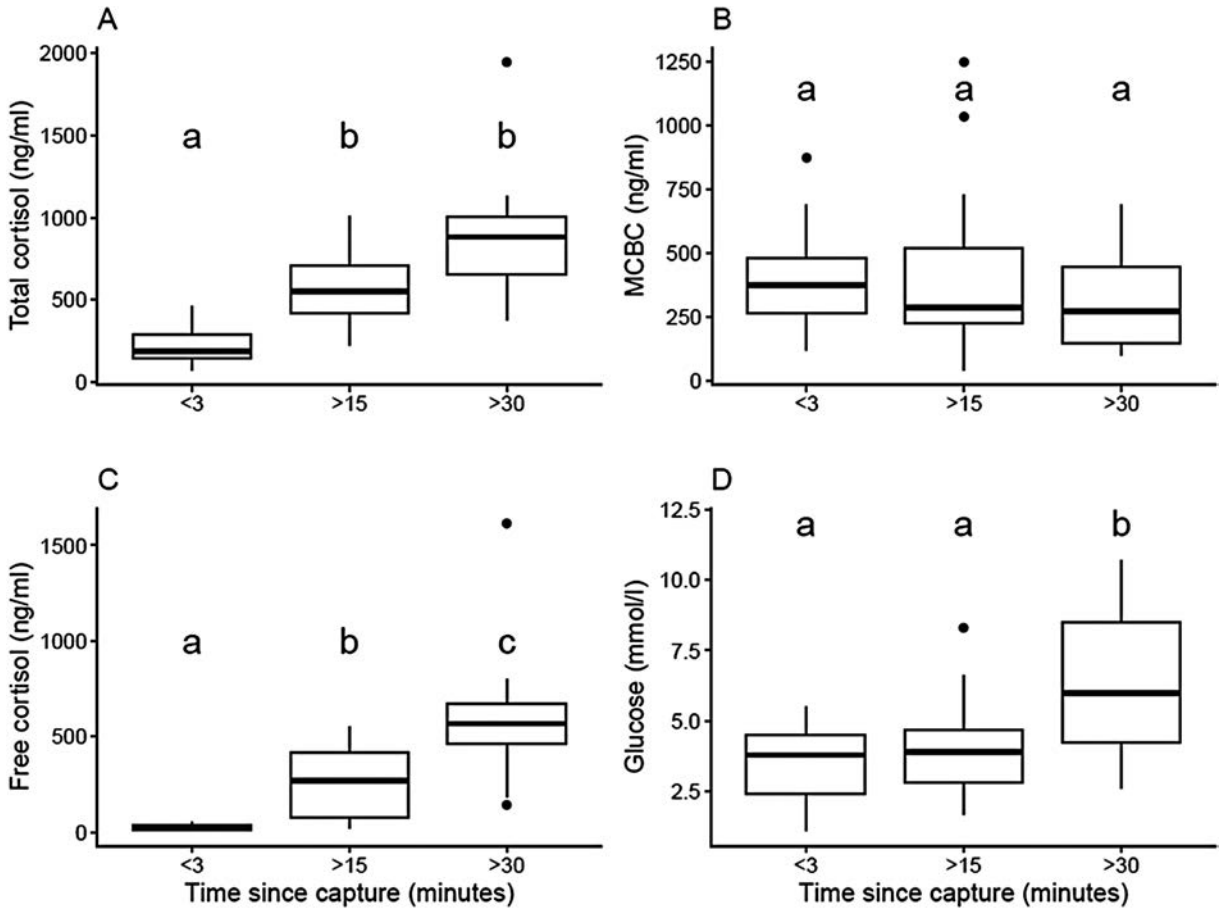

Fig. 2.—Stress-axis measures of groups of little brown bats (*Myotis lucifugus*) sampled at baseline (< 3 min), 15–30 min, and > 30 min after capture. Different lowercase letters denote groups that differ ($P < 0.05$) for each stress-axis measure, based on post-hoc tests. Boxplots represent the 25th–75th percentiles of the data, with error bars extending to the 10th and 90th percentiles, and dots representing data outside of these values. Solid lines represent the median value.

In the bats sampled later in the summer for the social holding condition study, there was an effect of sampling date on total cortisol and MCBC levels. Bats sampled in early July, during late pregnancy, had 1.4 times higher mean total cortisol levels ($P < 0.001$) and 2.0 times higher mean MCBC levels than those sampled in late July during lactation/post-lactation. When accounting for holding condition, total cortisol levels and MCBC were significantly higher in early July ($P < 0.001$ for both measures; Table 2; Fig. 3A and B). There also was a marginal increase in blood glucose levels in bats sampled in late July ($P = 0.09$). However, there ultimately was no difference in free cortisol levels by sampling date ($P = 0.41$). When accounting for the variation due to sampling date, bats held in solitary conditions had lower total cortisol levels than those held in a group ($P = 0.03$; Fig. 3A). None of the other measures showed any differences based on holding condition ($P = 0.29$; Table 2; Fig. 3). We also tested the capture date and holding condition interaction because capture date had a strong effect on some of these stress-axis measures. However, there was no detectable interaction effect on any of the four response variables, nor did it improve the fit of the models based on AIC score. For example, in the total cortisol model, the interaction effect did not approach significance (ß = –0.007 ± 0.11, $P = 0.94$) and the AIC with the interaction effect was 42 and without the interaction effect was 37. We therefore did not include the interaction effect in the final models (Table 2).

Table 2.—Model output of linear mixed effect models testing the effects of holding condition and sampling date on stress-axis measures of captured adult female little brown bats (*Myotis lucifugus*) from Yukon, Canada. Holding condition represents solitary housed, relative to group housed as the intercept. Sampling date are late July, relative to early July as the intercept. Bat origin colony was used in all models as a random effect.

Response variable	*n*	Parameter	Estimate ± SE	*t* value	*P* value
Total cortisol (log ng/ml)	115	Holding condition	–0.12 ± 0.05	–2.16	0.03
		Sampling date	–0.27 ± 0.06	–4.65	<0.001
MCBC (log ng/ml)	115	Holding condition	–0.06 ± 0.07	–0.98	0.33
		Sampling date	–0.28 ± 0.07	–4.18	<0.001
Free cortisol (log ng/ml)	109	Holding condition	–0.07 ± 0.14	–0.48	0.63
		Sampling date	0.13 ± 0.15	0.82	0.41
Glucose (log mmol/l)	124	Holding condition	–0.04 ± 0.04	–1.07	0.29
		Sampling date	0.07 ± 0.04	1.72	0.09

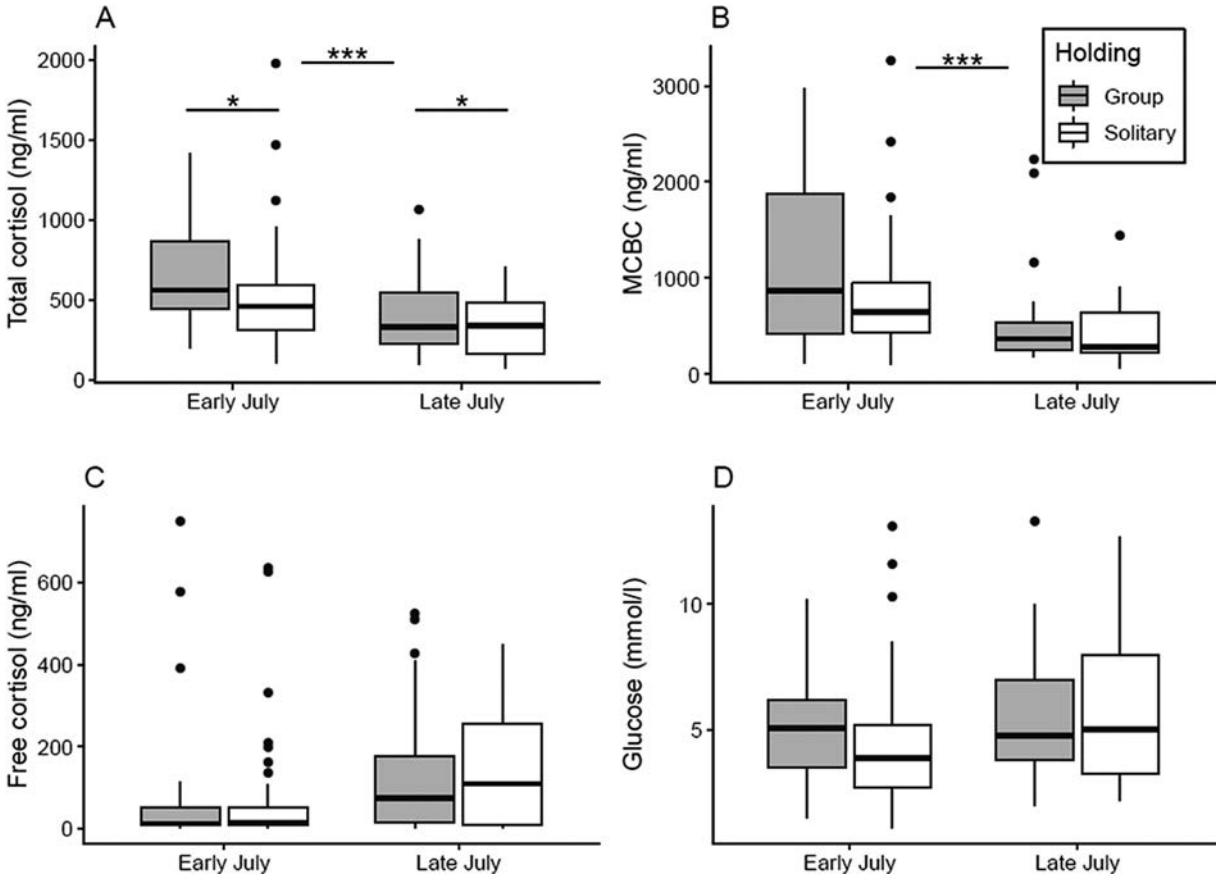

Fig. 3.—Stress-axis measures of little brown bats (*Myotis lucifugus*) held in groups or held solitarily, along with sampling date. Significance of the holding condition and sampling date is noted (*$P < 0.05$, ***$P < 0.001$), with A) a significant effect of holding condition ($P < 0.05$) on total cortisol levels and B) a significant effect of sampling date ($P < 0.001$) on total cortisol levels and C) on MCBC levels. Boxplots represent the 25th–75th percentiles of the data, with error bars extending to the 10th and 90th percentiles, and dots representing data outside of these values. Solid lines represent the median value.

Discussion

Our results highlight several key points about the little brown bat stress-axis. First, capture and confinement elevates cortisol levels rapidly in little brown bats. This is consistent with Reeder et al. (2004), who demonstrated that bats sampled 15 min after capture had significantly higher total cortisol levels than those sampled in ≤ 3 min. Our results expand on the findings of Reeder et al. (2004), as we found that there is a marginal difference in total cortisol between bats held 15–30 min and those held for > 30 min. Furthermore, because we measured MCBC levels during capture, we were able to document a strong increase in free cortisol levels among the baseline, 15–30 min, and > 30 min capture groups (Fig. 2C). This stronger difference in free cortisol likely is due to slight drops in MCBC (not statistically significant) during the time after capture that nonetheless result in an increase in free cortisol as total levels increase. While stressors are known to reduce CBG levels in some species, this change tends to occur across a longer timescale (4–24 h). In contrast, in some other species, there is no detectable change in CBG in response to stressors (Marti et al. 1997; Cyr et al. 2007; Delehanty and Boonstra 2009). It is possible that after a longer period, a stronger decline in MCBC levels would have been detected in little brown bats. Alternatively, animals between groups may have experienced the same amount of stress, but stress measures still were changing and provide a snapshot of the changing process during each time point. If so, this likely would be the case for any study that measures stress response over relatively short time intervals. Regardless, our MCBC data indicate that the stress-response had not peaked at 15–30 min, and that capture acts as a persistent stressor for bats held > 30 min.

In addition to prolonged elevation of GCs, we found that after 30 min of capture, little brown bats had higher blood glucose levels than those sampled at baseline or within 15–30 min. This timeframe is similar to some other mammals. For example, in variable flying foxes, blood glucose was higher after 15 min handling relative to baseline (Widmaier and Kunz 1993). Live-captured brown lemmings (*Lemmus trimucronatus*) also showed increases in blood glucose by 30 min, particularly for juveniles (Fauteux et al. 2017). However, the change we observed in glucose for little brown bats is more rapid than that reported in some other small mammals. Interestingly, in flying squirrels (*Glaucomys* spp.), there was no difference in blood glucose levels in samples collected < 3 min and after 30 min of capture (Desantis et al. 2016). Similarly, red squirrels (*Tamiasciurus hudsonicus*) showed no difference in blood

glucose levels between individuals sampled in < 3 min after capture compared to those sampled up to 4.5 h later (Bosson et al. 2012). The fact that glucose levels were elevated in little brown bats so quickly after capture further emphasizes the need to minimize holding time after capture for this species. This is particularly true for bats arousing from daily torpor and beginning their nightly foraging activity. Individuals captured after an initial foraging bout may respond differently to being captured than individuals in our study. Although we did not test other downstream effects of GC in this study (e.g., packed cell volume, white blood cell count), the fact that glucose was mobilized quickly has implications for future physiological work for little brown bats that looks at any measure in the blood that is influenced by GC action, such as measures of immune function. Thus, time held after capture is an important consideration for physiological work in this species in general.

We detected an effect of sampling date on some stress-axis measures. While there were no differences on any of these measures in samples collected in late May and mid-June, when all females sampled were presumed to be in early pregnancy, there was a sampling date effect on total cortisol and MCBC levels in early July compared to late July. This effect likely is driven by reproductive phenology. Total cortisol levels in little brown bats are higher in late pregnancy than they are during early pregnancy or lactation (Reeder et al. 2004). Female bats we sampled were in late pregnancy during the early July sampling period, and those in late July were lactating. Our observation of higher total cortisol levels in early July relative to late July likely is a result of females in our study being in different reproductive stages. Another possibility is that the early July bats were exposed to a weather condition or another environmental stressor around the time of sampling that could have driven an increase in total cortisol levels. However, the higher MCBC levels in early July support the idea that pregnancy, not an environmental stressor, was driving the total cortisol increase during this time. Exposure to stressors tends to decrease CBG levels over time (Boonstra 2005), whereas pregnancy increases CBG levels in many mammals due to estrogen-stimulated hepatic secretion of CBG during pregnancy (Edwards and Boonstra 2018). We recommend that in future work with bats, seasonality, in particular with reference to region-specific reproduction, be considered when assessing physiological measures.

Finally, solitary or group holding during capture did not have a particularly strong effect on stress-axis measures in little brown bats. Solitarily held bats had lower total cortisol levels than group held bats, but no differences in MCBC, free cortisol, or blood glucose levels. At least in this timeframe, there did not appear to be any stress of solitary holding conditions or separation from the colony above and beyond capture stress. Similarly, because little brown bats are socially tolerant naturally (Fenton and Barclay 1980), it is not surprising that group holding conditions do not act as a severe stressor. There may be a sex effect in this study, as all bats sampled were females. Female little brown bats roost in large maternity colonies throughout the summer, while males tend to roost separately or in smaller groups (Fenton and Barclay 1980; Randall et al. 2014). Males in the summer therefore may be less socially tolerant than females and perhaps would show a greater stress response when held with conspecifics in a group. It also should be noted that the bats were trapped at colony sites, and therefore likely held with familiar individuals, whereas if they were trapped at swarming sites, there would be a greater likelihood of being held with unfamiliar individuals. Being held with unfamiliar individuals could possibly result in a greater stress response than holding with familiar individuals, where social dynamics are established. However, at least during these relatively brief periods of capture and holding at colony sites, group holding conditions do not severely affect female little brown bat stress-axis measures.

Our study supports recovery initiatives for little brown bats by adding new information to the limited literature on stress responses in live-captured bats (e.g., Widmaier et al. 1994; Reeder et al. 2004), and more generally to that for small mammals (e.g., Fletcher and Boonstra 2006; Delehanty and Boonstra 2009; Bosson et al. 2012; Desantis et al. 2016). Importantly, the population-level effects of stress as a result of being captured require further investigation, particularly when individuals may be captured repeatedly over one or more years. In the interim, our findings suggest that capture time for little brown bats should be minimized, with bats handled and if possible released within 30 min of capture, to reduce stress. Reproductive phenology influences total and free cortisol in this species, so reproductive state must be accounted for when assessing stress axis measures in this species. Finally, although effects of holding conditions were weaker than those of capture time, solitary holding conditions did not appear to increase stress. This supports holding bats individually after capture, instead of in groups, to reduce risk of pathogen and parasite transmission.

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Conflict of Interest

None declared.

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